# EVALUATION OF SUGARCANE CLONES IN THE CP-CULTIVAR PROGRAM FOR RESISTANCE TO *PUCCINIA KUEHNII*, THE PATHOGEN OF ORANGE RUST

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**KEYWORDS*: Puccinia kuehnii*, Orange Rust, Sugarcane, Inoculation Techniques.**

**Abstract**

SINCE THE DETECTION of sugarcane orange rust in Florida in 2007, there has been a program to identify resistant clones in the Canal Point (CP) Cultivar Development Program. Both natural infection and artificial inoculation techniques have been used. Although natural infection requires less labour, results are dependent on the conduciveness of environmental conditions. A whorl inoculation method initially developed for brown rust has now been used annually for approximately 1700 clones that are in yield trials (Stages II through IV) in the CP Cultivar Development Program. Symptoms are rated using two different systems. Natural infection is rated using the rust severity level (percentage of leaf area affected), while whorl-inoculated plants are rated on the presence and level of pathogen sporulation. The two methods have demonstrated good agreement (correlations, $r^2$ = 0.72–0.95), although higher numbers of genotypes are rated susceptible by the whorl inoculation technique.

## Introduction

Orange rust, caused by *Puccinia kuehnii*, E.J. Butler (Ryan and Egan, 1989) is an airborne disease and rust spores can easily be transported over long distances, even between continents (Purdy *et al*., 1985). Long-distance transport of fungal spores is further aided by upward air currents (Brown and Hovmoller, 2002), such as those commonly generated/ created by preharvest burns (Mims and Mims, 2004).

Orange rust was confirmed in Florida in 2007, a first report for this disease in the western hemisphere (Comstock *et al*., 2008). Since then it has been recorded in many Central and South American countries (Ovalle *et al*., 2008; Pérez-Vicente, *et al*., 2009; Chavarría *et al*., 2009; Cadavid *et al*., 2012). Several widely-planted commercial clones, namely CP 80-1743, CP 88-1762, and CP 89-2143, exhibited susceptibility to orange rust in Florida, resulting in yield and economic losses. Fungicides, particularly strobilurins and triazoles, have demonstrated efficient control of orange rust in Florida (Raid *et al*., 2009).

Genetic resistance is favoured over the use of fungicides because of real or perceived environmental and health risks due to residue persistence (Gullino and Kuijpers, 1994), and the need to minimise production costs. Additionally, there is a risk of resistance to the fungicides building up in pathogen populations (Bäumler *et al.*, 2003). The Canal Point (CP) cultivar development program (cultivar program) has made it a priority to produce orange rust resistant cultivars because of the effects of orange rust on yield.

To select orange rust resistant clones, there is a need for an effective, reproducible method for screening genotype reactions to the rust pathogen. Natural infection is a useful and relatively cheap method for assessing resistance, but it has several disadvantages. Disease development is unreliable as it depends on the prevailing weather conditions and on the presence of sufficient

inoculum. For this reason, a whorl inoculation technique (Sood *et al*., 2009) was evaluated for orange rust. The advantages of artificial inoculation include: 1.) Uniform exposure of all plants under favourable conditions for the disease, 2.) Optimal pathogen inoculation concentration, and 3.) Appropriate check clones to assess inoculum and environmental conditions for disease development. The main objective of the CP cultivar program is to select for effective resistance to orange rust and other economically important diseases in high yielding sugarcane clones.

## Materials and methods

### Screening clones for orange rust resistance

### Canal Point cultivar development program

**Table 1**—Sugarcane field station cultivar development program.

| Stage | Population |
|---|---|
| Crossing | 400–600 crosses producing about 500 000 true seeds |
| Seedlings | 80 000–100 000 |
| Stage I (First clonal trial) | 10 000–15 000 |
| Stage II (Second clonal trial) | 1500 clones (varieties). |
| Stage III (First replicated test-off station) | 135 varieties |
| Stage III increase | 40 varieties selected from the Stage III to increase seed cane |
| Stage IV (Final replicated test-off station) | 16 varieties |
| Seedcane increase and distribution | Usually 6 or fewer varieties |

The CP cultivar program assigns series by year, followed by a four digit number starting with 1001 to clones advanced from Stage 1 to Stage 2. For example, clones in Stage 1 in the 2008 advanced to Stage 2 in 2009 were assigned the series name CP 08.

### Natural infection

In both the seedling and Stage 1 selections, rust resistance is based only on natural infection. Selection of rust resistant clones in Stage 2 and later selection stages of the breeding program are based on both natural infection and the leaf whorl inoculation technique.

The rating scale for natural infection consists of five classes: 0 (resistant), 1 (moderately resistant), 2 (moderately susceptible), 3 (susceptible), and 4 (highly susceptible) determined primarily on the bases of size and number of uredia.

### Leaf whorl inoculation

The method used for leaf whorl inoculations is described by Sood *et al*. (2009). Screening for orange rust using the leaf whorl inoculation method began in April–May 2009. In 2009, CP 08 series clones were inoculated in Stage 2 of the Canal Point breeding program and resistant clones were selected based on natural infection and the leaf whorl inoculation test. In 2012, plants in Stage 2 (CP 11 series), Stage 3 (CP 10 series), Stage 3 increase (CP 09 series) and Stage 4 (CP 08 series) were inoculated in April and were rated in May using leaf whorl inoculation.

Plants to be inoculated were marked by cutting off the distal third of the tips of the upper-most leaves so that inoculated stalks could be easily identified at the time of rating. A 0.5 mL aliquot of urediniospore suspension containing $10^4$ urediniospores/mL and 0.002% 1-nonanol (Sigma-Aldrich, St. Louis) was placed separately inside the leaf whorls of three individual stalks per replicate using a repeater pipette (Nichiryo model 8100, Tokyo, Japan).

Care was taken not to disturb the plants after inoculation, to ensure the inoculum was retained in the leaf whorl. Symptoms appeared on leaves (of susceptible clones) as a band of pustules after they emerged from the whorl.

Following a 4-week incubation, rust symptoms were rated on 0–4 scale, with 0 = no symptoms, 1 = chlorotic flecks, 2 = orange-brown lesions, 3 = 1–5 pustules with sporulation (production of urediniospores), and 4 = 5 or more pustules with sporulation that coalesces leading to leaf necrosis. Mean ratings across replicates were calculated. An average rating of 0–1 was considered 'resistant', 2 was considered 'moderately resistant', 3 'moderately susceptible', and 4 'susceptible'. Standard errors for means were determined and data analysed using the REG procedure and SAS software (version 9.1; SAS Institute Inc., Cary, NC).

## Results

Ratings for orange rust recorded after leaf whorl inoculation of clones in Stage 2, Stage 3, Stage 3 increase and Stage 4 were compared with rust ratings obtained after natural infection for the same clones. Ratings showed a significant ($P$ = 0.05) correlation ($r^2$=0.72–0.95) between natural infection and leaf whorl inoculation. However, some clones exhibiting a susceptible rating (ratings 3 and 4) after the leaf whorl inoculation did not exhibit a susceptible reaction with natural infection.

The percentage of rust resistance and susceptibility ratings differed between the two methods of inoculation and between the stages (Tables 2 and 3). More clones were rated susceptible using the leaf whorl inoculation technique for all the stages, ranging from 4% more in Stage 3 to 18% more in Stage 2. Stage 2 had higher percentages of resistant as well as susceptible clones than Stage 3 and Stage 3 increase with both screening methods. In contrast, later stages exhibited a higher percentage of moderately resistant clones than in Stage 2 (Tables 2 and 3). Whorl inoculation showed that Stage 4 had a higher percentage of resistant clones than the other stages (Table 3).

**Table 2**—2012 orange rust rating of clones based on natural infection.

| Rating | Percent clones | | | |
|---|---|---|---|---|
| | CP 11 Series in Stage 2 | CP 10 Series in Stage 3 | CP 09 Series in Stage 3 increase | CP 08 Series in Stage 4 |
| Resistant | 69 | 47 | 60 | 65 |
| Moderate resistant | 2 | 23 | 26 | 30 |
| Moderate susceptible | 6 | 18 | 7 | 0 |
| Susceptible | 23 | 12 | 7 | 5 |

**Table 3**—2012 orange rust rating of clones based on leaf whorl inoculation.

| Rating | Percent clones | | | |
|---|---|---|---|---|
| | CP 11 Series in Stage 2 | CP 10 Series in Stage 3 | CP 09 Series in Stage 3 increase | CP 08 Series in Stage 4 |
| Resistant | 45 | 41 | 43 | 50 |
| Moderate resistant | 8 | 24 | 26 | 30 |
| Moderate susceptible | 20 | 19 | 17 | 15 |
| Susceptible | 27 | 16 | 14 | 5 |

The CP 08 series were rated using both natural infection and leaf whorl inoculation in years 2009 to 2012. Also, the CP 09 Series clones were evaluated using both methods in 2011 and 2012 (Tables 4 and 5). Both methods were able to select resistant clones but the leaf whorl inoculation method was able to detect more susceptible clones than natural infection. Natural infection rust rating showed that the CP 08 series contained 85% resistants and 15% moderately resistant clones, with no susceptible clones (Table 4).

In contrast, leaf whorl inoculation of the CP 08 series clones found 46% resistant, 27% moderately resistant, 19% moderately susceptible and 8% susceptible (Table 5). The CP 08 series in Stage 2 had a higher percentage of moderately susceptible and susceptible clones than later stages (Tables 4 and 5). In general, Stage 2 had more resistant cultivars after natural infection (Table 4) than the other stages.

Leaf whorl inoculation rust rating of CP 08 series showed a gradual increase in total resistance from Stage 2 through Stage 4 (Table 5). In 2012 Stage 4, 50% of the clones were resistant and 30% of the clones were moderately resistant to orange rust. Results showed that a higher percentage of resistant CP 09 clones were advanced from Stage 3 to Stage 3 increase.

Unfortunately, in 2010 we were not able to inoculate the CP 09 series in Stage 2, and resistant cultivars based only on natural infection were advanced to 2011 Stage 3.

**Table 4**—Orange rust natural infection ratings of CP 08 and CP 09 Series in different Stages of the Canal Point Cultivar Program

| Rating | Percent clones | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | CP 08 Series | | | | CP 09 Series | | | CP 10 Series | |
| | 2009 in Stage 2 | 2010 in Stage 3 | 2011 in Stage 3 increase | 2012 in Stage 4 | 2010 in Stage 2 | 2011 in Stage 3 | 2012 in Stage 3 increase | 2011 in Stage 2 | 2012 in Stage 3 |
| Resistant | 79 | 61 | 85 | 65 | 77 | 48 | 60 | 75 | 41 |
| Moderate resistant | 11 | 32 | 15 | 30 | 9 | 34 | 26 | 7 | 24 |
| Moderate susceptible | 8 | 5 | 0 | 0 | 8 | 15 | 7 | 11 | 19 |
| Susceptible | 2 | 2 | 0 | 5 | 5 | 3 | 7 | 5 | 16 |
| Highly susceptible | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 |

**Table 5**—Orange rust leaf whorl inoculation rating of CP 08 and CP 09 Series in different Stages of the Canal Point breeding program.

| Rating | Percent clones | | | | | |
|---|---|---|---|---|---|---|
| | CP 08 Series | | | | CP 09 Series | |
| | 2009 in Stage 2 | 2010 in Stage 3 | 2011 in Stage 3 increase | 2012 in Stage 4 | 2011 in Stage 3 | 2012 in Stage 3 increase |
| Resistant | 49 | 40 | 46 | 50 | 35 | 43 |
| Moderate resistant | 16 | 28 | 27 | 30 | 23 | 26 |
| Moderate susceptible | 22 | 21 | 19 | 15 | 25 | 17 |
| Susceptible | 13 | 11 | 8 | 5 | 17 | 14 |

## Discussion

Higher numbers of clones exhibiting resistance with natural infection (Table 1) could be escapes and/or have different reaction to rust at the time of the rating. It is common to have little or no rust appearing on susceptible clones at various times of the year.

In 2009, 2010 and 2011, orange rust developed in late April and May, then tapered off during June. Thus, each year there have been times when orange rust assessments gave false resistance ratings based on natural infection.

Also, field observations show that under natural infection, clones and/or cultivars planted at different locations show varying rust reactions and severities, depending upon the time of rust development period (data not shown). Therefore, to gather better natural infection data, clones need to be rated several times during the orange rust development period at several locations.

Elevated numbers of resistant clones using natural infection in Stage 2 (Tables 2 and 4) appear to be escapes, since whorl inoculation identified some of them as susceptible and most of these whorl inoculation identified susceptible clones showed orange rust natural infection in following years (data not shown).

Higher percentage of susceptible clones after leaf whorl inoculation than natural infection (Tables 2 and 3) could be because in leaf whorl inoculation, clones were inoculated with a composite of spores collected from different locations and different varieties to include all possible variants.

Natural infection and leaf whorl inoculation ratings show that the percentage of resistant clones increased with selection stage at Canal Point (Tables 4 and 5). The lack of susceptible clones in the 2011 'Stage 3 increase' natural infection assessment could be due to low disease pressure / longer latent period. However, leaf whorl inoculation tests were able to identify susceptible clones (Table 5) probably because of more rapid disease development (within four weeks) associated with the use of optimal inoculum concentration of *P. kuehnii* urediniospores. Previous studies on *Puccinia hordei* suggest infection is quicker with shorter latent periods with increasing spore density (Baart *et al*., 1991; Teng and Close, 1978).

In summary, results show that the orange rust resistance cultivar selection process at Canal Point was able to increase the general resistance in clones in the selection program. An efficient, reproducible screening method for orange rust is important in order to develop resistant commercial cultivars. The leaf whorl inoculation technique appears to be an efficient technique for screening a large number of sugarcane clones for *P. kuehnii* resistance under field conditions. It also eliminates the need to wait for optimal natural infection conditions

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# ÉVALUATION DES CLONES DE CANNE À SUCRE DANS LE PROGRAMME DE DÉVELOPPEMENT DES CULTIVARS CP POUR LA RÉSISTANCE AU *PUCCINIA KUEHNII*, LE PATHOGÈNE DE LA ROUILLE ORANGÉE

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**MOTS-CLÉS: *Puccinia kuehnii*, Rouille Orangée, Canne à Sucre, Techniques d'Inoculation.**

**Résumé**

DEPUIS LA DÉTECTION de la rouille orangée de la canne à sucre en Floride en 2007, un programme a été mis en place pour identifier les clones résistants dans le programme de développement des cultivars de Canal Point (CP). L'infection naturelle, ainsi que des techniques d'inoculation artificielle ont été utilisées. Bien que l'infection naturelle nécessite moins de main d'œuvre, les résultats sont dépendents des conditions favorables et environnementales. Une méthode d'inoculation au niveau des verticilles, initialement développée pour la rouille brune, est maintenant utilisée tous les ans sur environ 1700 clones qui sont inclus dans les essais de rendement (Stades II à IV) dans le Programme de Développement des Cultivars CP. Les symptômes sont évalués en utilisant deux systèmes différents. L'infection naturelle est évaluée en se basant sur la gravité de la rouille (pourcentage de la surface foliaire affecté), tandis que les plants inoculés au niveau du verticille sont évalués sur la base de la présence et le degré de sporulation du pathogène. Les deux méthodes ont montré une bonne concordance (corrélations, r2 = 0,72-0,95), bien qu'un plus grand nombre de génotypes sont classés sensibles par la technique d'inoculation du verticille.

# EVALUACIÓN DE CLONES DE CAÑA DE AZÚCAR EN EL PROGRAMA CP, EN SU RESISTENCIA A *PUCCINIA KUEHNII*, PATÓGENO DE LA ROYA NARANJA

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**PALABRAS CLAVE**: ***Puccinia kuehnii*, Roya Naranja, Caña de Azúcar, Técnicas de Inoculación.**

**Resumen**

DESDE LA APARICIÓN en 2007 de la roya naranja en la Florida, se ha tenido un programa para identificar clones resistentes en el Programa de desarrollo de cultivares de Canal Point (CP). En la inoculación se ha utilizado tanto la infección natural como técnicas de inoculación artificial. Aunque la infección natural requiere menos mano de obra, los resultados dependen de la ocurrencia de condiciones ambientales favorables. El método de inoculación del espiral del cogollo,

desarrollado inicialmente para la roya café, se ha empleado anualmente en aproximadamente 1700 clones que se encuentran en los ensayos de rendimiento (Estados II a IV) del Programa de Desarrollo de Cultivares CP. Los síntomas se evaluaron mediante dos sistemas diferentes, dependiendo de si la infección fue natural, entonces se evaluó mediante el nivel de severidad de la roya (porcentaje de área foliar afectada), mientras que si las plantas fueron inoculadas en el espiral del cogollo, se clasificaron mediante la presencia y el nivel de esporulación del patógeno. Los dos métodos mostraron una buena concordancia (correlaciones, $r^2$ = 0.72 a 0,95), aunque se encontró un mayor número de genotipos susceptibles mediante la técnica de inoculación del espiral del cogollo.

## AVALIAÇÃO DE CLONES DE CANA-DE-AÇÚCAR NO PROGRAMA CP-CULTIVAR PARA DETERMINAÇÃO DE RESISTÊNCIA AO *PUCCINIA KUEHNII*, O PATÓGENO DA FERRUGEM ALARANJADA

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**Resumo**

DESDE A DETECÇÃO da ferragem alaranjada na Flórida em 2007, existe um programa para identificar clones resistentes no Programa de Desenvolvimento de Cultivares de Canal Point (CP). Foram utilizadas infecção natural e técnicas de inoculação artificial. Embora a infecção natural demande menos trabalho, os resultados dependem das condições ambientais. Um método de inoculação em espiral inicialmente desenvolvido para a ferrugem marrom está sendo usado anualmente para cerca de 1.700 clones em testes de produtividade (estágios II a IV) no Programa de Desenvolvimento de Cultivares de CP. Os sintomas são classificados utilizando-se dois sistemas diferentes. A infecção natural é classificada com o uso de severidade da ferrugem (porcentagem foliar afetada), ao passo que as plantas inoculadas em espiral são classificadas de acordo com a presença e o nível de esporulação do patógeno. Os dois métodos têm apresentado boa compatibilidade (correlações, $r^2$ = 0,72–0,95), embora um maior número de genótipos sejam classificados como suscetíveis pela técnica de inoculação em espiral.